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Sensitivity to DNA Damage Induced by Benzo(*a*)pyrene Diol Epoxide and Risk of Lung Cancer: A Case-Control Analysis[1]

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ABSTRACT

Levels of DNA adducts vary greatly *in vivo,* attributable to individual differences in enzymatic bioactivation of benzo(*a*)pyrene. We developed an assay to measure the levels of DNA adducts induced *in vitro* by benzo(*a*)pyrene diol epoxide (BPDE), a bioactivated form of benzo(*a*)pyrene. In this large molecular epidemiological study of lung cancer, we tested the hypothesis that the level of *in vitro* BPDE-induced adducts is associated with risk of lung cancer. This hospital-based case-control study included 221 newly diagnosed lung cancer cases and 229 healthy controls frequency matched on age, sex, ethnicity, and smoking status. Short-term cultured peripheral blood lymphocytes from each subject were exposed *in vitro* to BPDE (4 μM) for 5 h, and the ^{32}P-postlabeling method was then used to measure BPDE-induced DNA adducts in the host cells. Overall, the patients had significantly higher levels of BPDE-DNA adducts than did the controls (mean ± SD per 10^7 nucleotides, 93.2 ± 89.3 for cases *versus* 63.7 ± 61.1 for controls; $P = 0.001$). Univariate and multivariate logistic regression analyses were performed to calculate the crude and adjusted odds ratios and their 95% confidence intervals. When the median adduct level of controls (46/10^7 nucleotides) was used as the cutoff point, 64% of cases had higher levels (odds ratio, 2.15; 95% confidence interval, 1.39–3.33, adjusted for age, sex, ethnicity, body mass index, recent weight loss, pack-years smoked, smoking in the last 24 h, and family history of cancer). Stratified analyses showed consistently higher levels of BPDE-induced adducts in cases than in controls, regardless of subgroup of age, sex, ethnicity, body mass index, recent weight loss, pack-years smoked, smoking in the last 24 h, and family history of cancer. A significant dose-response relationship between the quartile levels of BPDE-induced DNA adducts and the risk of lung cancer was observed (trend test, $P < 0.001$). The significant association between the level of *in vitro* BPDE-induced DNA adducts and risk for lung cancer suggests that subjects very sensitive to BPDE-induced DNA damage may have a suboptimal ability to remove the BPDE-DNA adducts and so are susceptible to tobacco carcinogen exposure and, therefore, may be at increased risk of lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death for both men and women in the United States (1). Although cigarette smoking is the major risk factor associated with 80% of lung cancer cases, only 10–15% of heavy smokers ultimately develop lung cancer (2). This implies that genetic variation in sensitivity to carcinogen exposure may play an important role in the etiology of lung cancer. Therefore, using biomarkers to identify individuals at high risk is an important step toward cancer prevention.

Interindividual variation in susceptibility to chemical carcinogenesis depends on carcinogen metabolism and DNA repair. For example, the level of *in vivo* tobacco smoke-induced DNA adducts is determined by an equilibrium between the metabolism of tobacco carcinogens, such as BP,[3] and the rate of adduct removal by DNA repair enzymes. Both processes are modulated by genetic polymorphisms and epigenetic factors (3, 4). Biomarkers reflecting such susceptibility may therefore be useful for identifying high-risk individuals. Genotypic biomarkers for carcinogen activation, detoxification, and DNA repair are being used extensively to characterize genetic susceptibility to carcinogenesis (5, 6). However, the functional relevance of some of these polymorphisms has not been determined. DNA adducts induced by ultimate carcinogens such as BPDE are phenotypic markers that reflect cellular response to the exposure, including detoxification and DNA repair. Therefore, individuals who are sensitive to BPDE-induced DNA damage may have suboptimal DNA repair capacity. To measure such DNA repair capacity, we developed and reported an *in vitro*-induced DNA-adduct assay using cultured peripheral lymphocytes that are exposed *in vitro* to BPDE (7). To further investigate the utility of this assay as a biomarker for genetic susceptibility to lung cancer, we used the assay for a large epidemiological study of 221 lung cancer cases and 229 healthy controls. Here we report an association between the level of *in vitro* BPDE-induced DNA adducts and risk of lung cancer.

MATERIALS AND METHODS

Materials. As described previously (7), anti-BPDE was purchased from National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO) and was dissolved in tetrahydrofuran (Sigma Chemical Co., St. Louis, MO). The 5 mg/ml (16.7 mM) stock solution was further diluted to 1 mM for the working solution. All solutions were prepared in a dark room and kept at −20°C.

Study Population. The cases were patients with newly diagnosed, histopathologically confirmed lung cancer seen at The University of Texas M. D. Anderson Cancer Center between 1996 and 1999 with a participation rate of 77.4% upon contact. Most nonparticipants were too ill to participate. Healthy control subjects were recruited from a large managed-care organization in the Houston metropolitan area (Kelsey Seybold Clinic) during the same time period (8). These control subjects were first surveyed with a short questionnaire for willingness to participate in research studies and to provide data on smoking behavior and demographics. A computer database of more than 50,000 potential control subjects was used to identify eligible control subjects who were individually selected to match the case patients by age (±5 years), sex, ethnicity, and smoking status (8). Of those identified and contacted, 73.3% participated. Each eligible subject was then scheduled for an interview to collect information on demographic data and risk factors such as smoking history. A 30-ml blood sample was obtained from each participant for the laboratory assays. The exclusion criteria were prior chemotherapy or radiotherapy for the cases, prior cancer for the controls, and recent (within the last 6 months) blood transfusion for any participant. The study protocol was approved by the M. D. Anderson and Kelsey Seybold Clinic Institutional Review Boards, and informed consent was obtained from all study participants.

Histology and Clinical Stage of Cancer. Histopathology and clinical stage of cancer were obtained from the clinical records. NSCC was staged in

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[3] The abbreviations used are: BP, benzo(*a*)pyrene; BPDE, BP diol epoxide; NSCC, non-small cell carcinoma; SCC, small cell carcinoma; PHA, phytohemagglutinin; OR, odds ratio; CI, confidence interval; BMI, body mass index; GST, glutathione *S*-transferase.

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accordance with the 1997 revision of the International System for Staging Lung Cancer (9). SCC was divided into "limited" and "extensive" disease, based on the absence or presence, respectively, of distant metastases. For this analysis, stages I and II NSCC were defined as "limited disease," stage III NSCC and limited SCCs as "regional spread" (because most of the SCCs had mediastinal lymph node involvement at the time of diagnosis), and stage IV NSCC and extensive SCC as "extensive spread."

Blood Sample Collection, Cell Culture, and BPDE Treatment. Within 8 h (the same day) after the blood samples were drawn, they were processed for cell culture as described previously (7). Briefly, 1 ml of whole blood was inoculated into each of two T-25 flasks (containing 9 ml of standard RPMI 1640 supplemented with 15% fetal bovine serum and 112.5 μg/ml PHA) and incubated at 37°C for 72 h. One flask was used as the *in vivo* background adduct control, and one was used for BPDE treatment later. Because unstimulated lymphocytes have little nucleotide excision repair of BPDE adducts (10, 11), PHA stimulation was necessary. No statistical difference was observed in the blastogenic rates in response to PHA stimulation between cases and controls (71.1% ± 18.3 and 73.4% ± 23.5, respectively; $P = 0.238$). To measure differences in the levels of *in vitro* BPDE-induced DNA adducts in the host cells, BPDE (a final concentration of 4 μM; see Fig. 1 for the dose optimization) was added after 67 h of PHA stimulation, and the incubation was continued for 5 h, which was sufficient for adduct formation and removal because: (*a*) the level of induced adducts peaks in the host cells within 15 min of BPDE exposure; (*b*) nearly half of the damage is removed by the host cells within 5 h; and (*c*) the level of adducts remains virtually unchanged thereafter for 24 h (12). This short-term treatment was optimal and also necessary to minimize reduction in induced DNA adducts because of DNA replication during cell division. After 72 h of PHA stimulation, the cells were harvested. The cell pellets were washed three times with methanol:acetic acid (3:1, v/v) to remove free DNA released by dead cells and to fix viable cells. The cleaned cell pellets were then stored in a −20°C freezer until used for DNA extraction.

DNA Isolation and Adduct Analysis. DNA was extracted from the cell pellets from an equal number of cases and controls in batches. The cells were incubated in digestion buffer [10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% SDS, and 0.1 mg/ml proteinase) at 50°C for 12 h. The cell lysates were then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v). Each aqueous phase was transferred to a fresh tube and adjusted to 150 mM sodium acetate and 10 mM $MgCl_2$, and DNA was precipitated with three volumes of ethanol at −20°C for 12 h. The DNA pellets were dissolved in 0.01× SSC, digested with RNase A and RNase T1 at 37°C for 2 h, and extracted with an equal volume of phenol/chloroform/isoamyl alcohol. The DNA was then precipitated with ethanol and dissolved in 0.01× SSC, and DNA concentration was measured by spectrophotometry.

The nuclease P1-enhanced version of the ^{32}P-postlabeling assay (13) was used for the DNA adduct analysis, and 2.5 μg of DNA from each sample were used. All cells and DNA samples were coded during laboratory analysis; therefore, case-control status was not known by the laboratory personnel. The codes were broken at the time of data analysis. An internal standard DNA from mouse skin treated with dibenzo[*a,j*]acridine was included in each DNA sample to monitor the quality of enzymatic digestion, radioactive labeling, and chromatography. As demonstrated previously (7), because DNA replication (at least twice) occurred as the cells transmitted the cell cycles, the levels of background *in vivo* DNA adducts in untreated cells were negligible (100-fold lower) compared with the levels of *in vitro* BPDE-induced DNA adducts in treated cells. Therefore, only BPDE-treated cells were analyzed. The adduct levels determined by this assay in DNA sample duplicates were highly reproducible, with a coefficient of variance of <15%. The average of the duplicates was used for comparison.

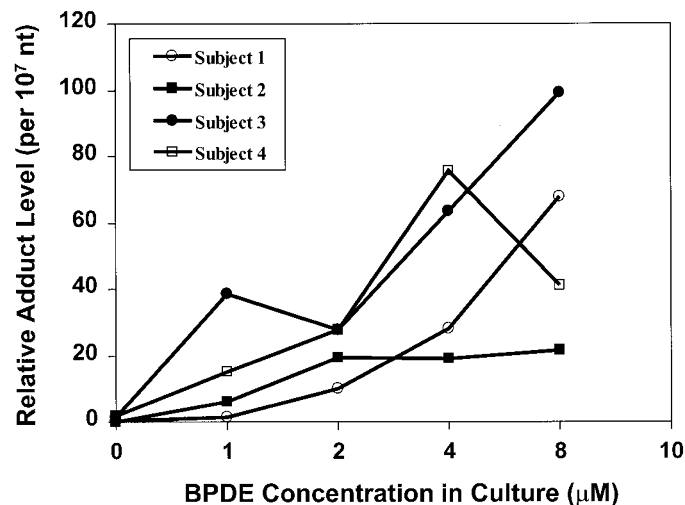

Fig. 1. Dose-response curve of relative adduct levels between concentrations of 0 and 8 μM BPDE in four blood samples. The relative adduct levels per 10^7 nucleotides (*nt*) increased as the BPDE dose (μM) increased up to the concentration of 4 μM for all samples.

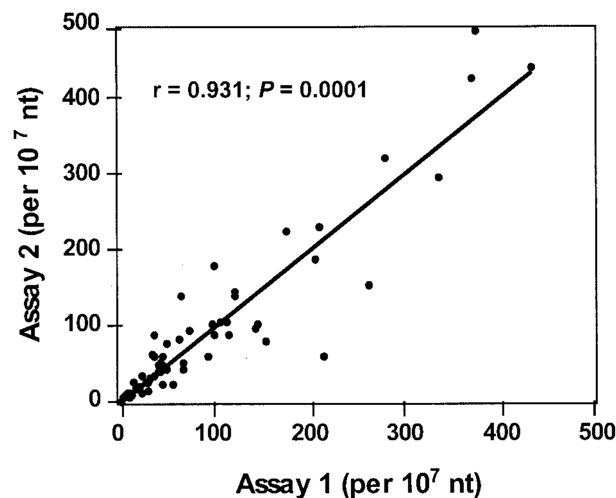

Fig. 2. Correlation between repeated assays for the relative level of BPDE-induced DNA adducts per 10^7 nucleotides (*nt*). The data from 58 samples showed a statistically significant correlation ($r = 0.931$; $P = 0.0001$) between the results of two repeated assays.

Assay Validation. The dose-response of four blood samples to 0–8 μM BPDE is shown in Fig. 1. In general, the BPDE-adduct level increased as the dose increased, although for one blood sample there was a fluctuation for subject 4 that suggested a possible toxic effect at the highest dose of 8 μM. Therefore, 4 μM was chosen for the all experiments in this report.

To assure the high quality of the data, ~13% of the samples ($n = 58$) were assayed twice. As demonstrated in Fig. 2, the repeated measurements were highly correlated ($r = 0.931$; $P = 0.0001$). These results justify measuring each sample only once for this study. In addition, the background (untreated) level of adducts in cultured cells from 16 controls had a mean of 2.29 (±SD, 2.29) per 10^7 nucleotides with a range of 0.15–9.28. Compared with a mean of >63.7/10^7 nucleotides for the treated 229 controls, the background levels were negligible. These results indicate that use of the adduct levels from BPDE-treated samples alone did not alter the statistical validity of the study (but did greatly reduce the assay cost).

Statistical Analysis. Sensitivity to DNA damage induced by BPDE was measured as the induced BPDE-DNA adduct levels and analyzed as a continuous variable. Logarithmic transformation was used in the exploratory analysis, and Student's *t* test was used to compare the differences in the mean adduct levels between groups using both transformed and untransformed data. BMI was calculated using the formula: weight in kg/(height in m)^2. Correlation analyses were performed to compare levels of BPDE-induced DNA adducts with selected host factors. To test for associations between the levels of induced adducts and cancer risk, the crude OR and the 95% CIs were calculated as estimates of relative risk by using both the median and quartile adduct levels in controls as the cutoff values. Multivariate logistic regression was used to calculate ORs and 95% CIs with adjustment for other covariates. All statistical analyses were performed with Statistical Analysis System Software (PC version 6.0; SAS Institute, Inc., Cary, NC).

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RESULTS

The demographic characteristics and select variables of the study subjects are summarized in Table 1. The cases and controls were well matched on age (51 and 50% >60 years, respectively), sex (52 and 58% males, respectively), ethnicity (86 and 85% non-Hispanic whites, respectively), and smoking status (90 and 91% ever-smokers, respectively). Although more cases reported to have smoked in the last 24 h (26%) and a family history of cancer in a first-degree relative (67%) compared with controls (20 and 63%, respectively), these differences were not statistically significant. However, significantly more cases (45%) had experienced weight loss in the previous year before diagnosis compared with the controls (21%).

The average ages (mean ± SD) were 60.3 ± 9.3 and 59.3 ± 10.8 for cases and controls, respectively, but the difference was not statistically significant ($P = 0.281$; Table 2). The average pack-years smoked were significantly higher (51.3 ± 36.9) among the cases than among the controls (43.6 ± 32.6; $P = 0.020$; Table 2). The cases also had a significantly lower BMI (25.8 ± 4.5) and experienced significant loss of weight (−0.02 ± 0.13 kg) in the last year than did the controls (27.2 ± 4.6 and 0.02 ± 0.13 kg, respectively; $P = 0.002$ and $P = 0.008$, respectively; Table 2). Furthermore, the levels of the induced DNA adducts were higher in cases (93.2 ± 89.3/10^7 nucleotides) than in controls (63.7 ± 61.1/10^7 nucleotides), and this difference was statistically significant ($P < 0.001$; Table 2). After logarithmic transformation of these levels, the log-adduct levels were 1.76 ± 0.50 for cases and 1.57 ± 0.56 for controls, and the difference remained statistically significant ($P < 0.001$; Table 2).

Stratified analyses revealed that the cases consistently had significantly higher levels of adducts than did the controls, regardless of subgroup of age, sex, ethnicity, smoking status, smoking in the last 24 h, weight loss, and family history of cancer (Table 3). The nonsignificant differences observed between cases and controls in other subgroups (Hispanics, Blacks, and never-smokers) were probably attributable to the small numbers of subjects (<25 for each subgroup). There was no evidence of interactions between these variables and the level of induced adducts. When subgroup differences in the levels of DNA adducts within cases and within controls were compared, respectively, the only statistically significant finding was for age within the control group ($P < 0.001$) but not within the case group ($P = 0.213$) because of larger variation (Table 3).

When the median (46/10^7 nucleotides) of controls' induced adduct levels was used as the cutoff value for calculating the OR, 65% of the cases were above this median level (Table 4), and the crude OR associated with a high adduct level was 1.82 (95% CI, 1.25–2.65). Logistic regression analysis revealed that having a high level of induced DNA adducts was an independent risk factor for lung cancer after adjustment for age, sex, smoking status, smoking in the last 24 h, BMI, weight loss, and history of first-degree relatives with cancer (OR, 2.23; 95% CI, 1.45–3.44). Furthermore, when the quartiles of DNA adduct levels in the controls were used as cutoff points to calculate the ORs, a significant dose-response relationship between increasing levels of induced DNA adducts and risk of lung cancer was evident (ORs of 1.11, 1.62, and 3.23; trend test, $P < 0.001$) after adjustment for age, sex, smoking status, smoking in the last 24 h, BMI, weight loss, and history of first-degree relatives with cancer (Table 4).

The tumors of the 221 cases included 95 adenocarcinomas, 50 squamous cell carcinomas, 45 NSCCs, 17 SCCs, and 14 large cell carcinomas (Table 5). There were 36 limited stages I-II, 67 regional spread, 55 extensive spread, and 63 unspecified. Although the cases in each subgroup (except those with <25 subjects) had significantly higher induced DNA adduct levels than did the controls, there were no significant differences in the levels of DNA adducts between different histopathological types and clinical stages (data not shown). For example, patients with the large cell carcinoma had the highest level of adducts (mean ± SD, 114.3 ± 98.0/10^7 nucleotides), whereas those with the SCCs had the lowest level of adducts (80.3 ± 73.4/10^7 nucleotides), but the difference was not statistically significant ($P = 0.161$). These findings suggest that *in vitro* induced DNA adducts are a constitutional rather than a tumor-specific marker.

Finally, to adjust for all possible confounders revealed by previous univariate analyses, a multivariate logistical regression model was fitted to include age, sex, ethnicity, pack years smoked, smoking in the last 24 h, BMI, weight loss, family history of cancer, and level of induced DNA adducts (Table 6). It is clear that the level of induced DNA adducts remained an independent predictor ($P < 0.001$) of lung cancer risk in the presence of other variables, although pack-years of smoking, BMI, and weight loss were also independent predictors of risk in the same model.

Table 2 *Means of selected variables in lung cancer patients and healthy controls*

	Cases (n = 221) Means ± SD	Controls (n = 229) Means ± SD	P^a
Age (yr)	60 ± 9.3	59.3 ± 10.8	0.281
(range)	(34–79)	(32–86)	
Pack years	51.3 ± 36.9	43.6 ± 32.6	0.020
	(0–192)	(0–189)	
BMI[b]	25.8 ± 4.5	27.2 ± 4.6	0.002
(range)	(14.7–38.4)	(17.8–45.6)	
Weight change (kg)[c]	−0.02 ± 0.13	0.02 ± 0.13	0.008
(range)	(−43–86)	(−55–60)	
DNA adducts per 10^7 nt[d]	93.2 ± 89.3	63.7 ± 61.1	<0.001
	(0.98–681.9)	(0.12–464)	
Log-adduct levels	1.76 ± 0.50	1.57 ± 0.56	<0.001
	(−0.01–2.83)	(−0.92–2.67)	

[a] Two-sided Student's *t* tests.
[b] BMI, weight in kg/[height in m]^2.
[c] There were 26 cases and 10 controls for whom we did not have information on BMI and weight loss.
[d] nt, nucleotides.

Table 1 *Distribution of selected variables in lung cancer patients and healthy controls*

	Cases (n = 221)		Controls (n = 229)		
	No.	%	No.	%	P^a
Age (yr)					
≤60	109	49	114	50	
>60	112	51	115	50	0.922
Sex					
Male	114	52	133	58	
Female	107	48	96	42	0.167
Ethnicity					
Non-Hispanic white	191	86	196	85	
Hispanic	8	4	13	6	
Black	22	10	20	9	0.546
Smoking					
Never	21	10	20	9	
Former	85	38	110	48	
Current	115	52	99	43	0.117
Smoked in previous 24 h					
No	164	74	184	80	
Yes	57	26	45	20	0.120
Weight loss in previous year[b]					
No	121	62	181	83	
Yes	74	38	38	17	0.001
Cancer in first-degree relatives					
No	72	33	85	37	
Yes	149	67	144	63	0.313

[a] χ^2 test for the distribution between cases and controls.
[b] There were 26 cases and 10 controls for whom we did not have information on BMI and weight loss.

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Table 3 *Levels of BPDE-induced adducts in lung cancer patients and healthy controls stratified by select variables*

	Cases (n = 221)		Controls (n = 229)		
	No.	Means ± SD	No.	Means ± SD	P^a
Age (yr)					
≤60	109	100.8 ± 94.6	114	77.6 ± 64.3	0.032
>60	112	85.8 ± 83.6	115	50.0 ± 54.7	<0.001
Sex					
Male	114	100.1 ± 89.1	133	62.6 ± 61.6	<0.001
Female	107	85.8 ± 89.4	96	65.4 ± 60.8	0.056
Ethnicity					
Non-Hispanic white	191	94.2 ± 91.1	196	61.8 ± 61.5	<0.001
Hispanic	8	93.5 ± 52.8	13	67.8 ± 62.1	0.326
Black	22	84.4 ± 87.1	20	80.0 ± 56.8	0.846
Smoking					
Never	21	103.7 ± 101.6	20	78.7 ± 82.6	0.391
Former	85	87.1 ± 75.0	110	59.0 ± 60.4	0.004
Current	115	95.8 ± 97.0	99	66.0 ± 56.9	0.006
Smoked in previous 24 h					
No	164	82.9 ± 75.8	184	65.9 ± 64.1	0.024
Yes	57	122.7 ± 116.0	45	54.4 ± 48.9	<0.001
Weight loss in previous year[b]					
No	121	86.5 ± 82.3	181	63.6 ± 60.8	0.006
Yes	74	110.3 ± 106.1	38	57.2 ± 49.9	0.004
Cancer in first-degree relatives					
No	72	95.7 ± 83.5	85	66.3 ± 71.5	0.019
Yes	149	92.0 ± 92.3	144	62.2 ± 54.3	<0.001

[a] Two-sided Student's t test for the differences between cases and controls.
[b] There were 26 cases and 10 controls for whom we did not have information on BMI and weight loss.

DISCUSSION

Lung cancer is the paradigm of a disease attributable to the interaction between genes and environmental exposure. Although cigarette smoking is the major risk factor for lung cancers, individual susceptibility to tobacco carcinogenesis plays a role in the etiology of lung cancer. Molecular epidemiological approaches to identify individuals at high risk, especially those who have difficulty in quitting smoking, are an important step in cancer prevention. Biomarkers being used in molecular epidemiological studies include genetic polymorphisms in carcinogen-metabolizing enzymes (5, 6), DNA repair genes (14), DNA damage (such as DNA adducts; Refs. 15 and 16), chromosome abnormalities (17–18), and functions of repair enzymes (7, 19, 20). In general, the level of DNA adducts *in vivo* is the integrated outcome of carcinogen activation and detoxification reactions and DNA repair. Therefore, the level of DNA adducts provides more information on cellular responses to carcinogen challenge than do individual enzyme activity or genetic polymorphisms. Thus, we developed an *in vitro* BPDE-induced DNA adduct assay (7) that measures a different end point in the same treated cells used in the BPDE-induced mutagen sensitivity assay, in which induced chromatid breaks are quantified (11). In this large molecular epidemiological study, we provide supporting evidence that sensitivity to DNA damage induced by BPDE (expressed as increased levels of *in vitro* BPDE-induced DNA adducts) is an independent risk factor for lung cancer. The consistent statistically significant difference in the levels of *in vitro* BPDE-induced DNA adducts between cases and controls suggests that this assay may be useful for providing quantitative information for assessment of individual susceptibility to environmental carcinogens.

Because BP is an abundant carcinogen present in cigarette smoke, sensitivity to BPDE may have a significant implication in risk assessment of tobacco-related cancers. Because BPDE, the active form of BP, is used in this assay, the main determinants of the level of induced adducts are carcinogen detoxification and DNA repair capacities of the test cells. Although the initial level of BPDE-induced DNA adducts was not measured in this study (because of technical difficulty when a large number of samples are tested), DNA binding of BPDE usually peaks within 15 min of exposure (12). Because we previously reported a significant correlation between reduced DNA repair capacity and higher level of BPDE-induced DNA adducts (21), we believe that variation in cellular DNA repair capacity may be the major underlying mechanism for the difference in *in vitro* induced DNA-adduct levels between cases and controls.

Early age of onset of disease is a hallmark of genetic predisposition, which can result in high sensitivity to low levels of exposure (22). Indeed, our results showed that younger cases tended to have higher levels of BPDE-induced DNA adducts than older cases and controls, although there was no clear evidence of interaction between age and level of the adducts. The significant high level of BPDE-induced

Table 4 *Logistic regression analysis of BPDE-induced adducts in lung cancer cases and healthy controls*

	Cases (n = 221)		Controls (n = 229)		Crude	Adjusted[a]
	No.	%	No.	%	OR (95% CI)	OR (95% CI)
BPDE adducts/10^7 nt[b] (by median)						
<46	78	35	114	50	1.00	1.00
≥46	143	65	115	50	1.82 (1.25–2.65)	2.23 (1.45–3.44)
BPDE adducts/10^7 nt (by quartiles)						
≤22	35	16	56	25	1.00	1.00
23–46	43	19	58	25	1.25 (0.70–2.24)	1.11 (0.58–2.12)
47–85	52	24	58	25	1.44 (0.83–2.48)	1.62 (0.88–2.97)
>85	91	41	57	25	2.62 (1.52–4.55)	3.23 (1.71–6.09)
Trend test					$P < 0.001$	$P < 0.001$

[a] Adjusted for age, sex, ethnicity, pack years smoked, smoking in the last 24 h, BMI, weight loss, and history of cancer in first-degree relatives. However, there were 26 cases and 10 controls for whom we did not have information on BMI and weight loss.
[b] nt, nucleotides.

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Table 5 *Level of BPDE-induced adducts by tumor histology and clinical stage in lung cancer cases*

	Number	Adduct levels (Mean ± SD)	P^a
Controls	229	63.7 ± 61.1	Reference
Cases	221	93.2 ± 89.3	<0.001
By histology			
Adenocarcinoma	95	101.2 ± 86.0	<0.001
Squamous cell carcinoma	50	80.3 ± 73.4	0.095
NSCC	45	87.3 ± 109.5	0.043
SCC	17	84.8 ± 87.3	0.185
Large cell carcinoma	14	114.3 ± 98.0	0.004
By clinical stage			
Limited	36	101.2 ± 97.4	0.002
Regional spread	67	99.5 ± 101.4	<0.001
Extensive spread	55	87.6 ± 78.3	0.015
Unspecified	63	86.8 ± 80.9	0.014

[a] Two-sided Student's *t* tests using the controls' value as the reference.

DNA adducts observed in younger cases suggests a reduced DNA repair capacity in young cancer patients, as reported previously (23). It is documented that women have higher risks of developing smoking-related cancer than men at similar levels of exposure (24). However, we did not find a significant sex difference in the sensitivities to BPDE-induced DNA damage.

In a pilot study of lung cancer, we found a tendency for higher levels of BPDE-induced adducts in nonsmoking cases than smoking cases (7). This trend was observed in the present study as well. Although the difference was not statistically significant, possibly because of the small number of nonsmokers studied, this trend suggests the importance of genetic background in cancer risk among individuals with no or low levels of carcinogen exposure. An early study showed that younger lung cancer patients with a family history of cancer had a significantly higher level of DNA adducts in their monocytes exposed *in vitro* to BP than did patients with no family history (22). This difference suggests a carcinogen sensitivity-related genetic predisposition to lung cancer. However, our study did not reveal any significant difference in the levels of BPDE-induced DNA adducts between individuals with or without family history of cancer. This observation suggests that genetic differences in biological functions other than nucleotide excision repair are also involved in inherited predisposition to lung cancer.

Although this *in vitro* BPDE-induced adduct assay may be useful in risk assessment of lung cancer, it has some limitations. One is the use of lymphocytes, the repair of which may not reflect that of lung epithelial cells. However, it has been demonstrated that the levels of smoking-induced DNA adducts in lymphocytes and lung are significantly correlated (25). Therefore, lymphocytes are a relevant and very accessible surrogate tissue for the lung. In this study, we assumed that if this assay measures DNA repair capacity that is genetically determined (as seen in xeroderma pigmentosum) and even tissue specificity exists, the individuals' levels of *in vitro* BPDE-induced adducts can be compared in the same type of tissue such as lymphocytes. Nevertheless, these assumptions could not be validated in this study. Although the BPDE-induced adduct level is probably determined by the host cells' DNA repair capacity, the fact that we did not measure the initial level of BPDE-induced adducts excludes the possibility of precisely measuring the repair rate. Furthermore, the large variation in the measurements suggests that this assay should be used for research purposes rather than diagnosis.

Another limitation of this study is that it was a retrospective study; therefore, we cannot rule out that there was a systemic effect on the level of BPDE-induced adducts in lung cancer patients. A tested case-control study would validate the findings in this study. Although it is possible that smoking and overall sickness of the lung cancer patients lowered their DNA repair levels, our detailed analyses of the effect of smoking and weight loss as well as disease stage did not support this possibility. The uniform concentration and time of *in vitro* BPDE exposure, uniform assay conditions, and the high level of BPDE-induced adducts in the treated cells measured by the sensitive ^{32}P-postlabeling method also minimize the possibility of artifacts in the measurements. Our consistent findings of statistically significant differences between relatively large numbers of cases and controls and the dose-response relationship between the levels of BPDE-induced adducts and risk of lung cancer are probably not attributable to chance. Furthermore, the findings in this study are consistent with those from another plasmid-based transfection (host-cell reactivation) assay we reported recently, in which consistently poorer repair of BPDE-induced adducts in the plasmids was evident in lung cancer patients compared with controls (26). These two assays should be complementary in defining DNA repair capacity in that the *in vitro* BPDE-induced adduct assay measures the repair of direct damage to genomic DNA, and the host-cell reactivation assay measures the repair of fixed damage to foreign (plasmids) DNA that have been transfected into the cells (27). The consistent results of these two assays in lung cancer patients further strengthen biological plausibility of our preliminary findings.

There is a growing body of evidence that reduced DNA repair is associated with increased cancer risk. Individuals with defective DNA repair have a >1000-fold increased risk for developing cancer as seen in xeroderma pigmentosum patients with skin cancer who have defective nucleotide excision repair genes (28); patients with hereditary nonpolyposis colon cancer who have defective mismatch repair genes (29); and patients with breast cancer who have defective *BRCA1* and *BRCA2* (30). This etiological association between deficient DNA repair and increased cancer risk is also supported by evidence from studies of sporadic cancers of the skin (23), lung (26), colon (31), and breast (32). This study presented the analysis of the main effect of the level of *in vitro* BPDE-induced DNA adducts as an independent risk factor. Because increased cancer risk is well documented in patients with genetically inherited defective DNA repair, our findings are biologically plausible. However, this assay alone may not provide a risk profile as complete as is needed in risk assessment. Further analyses of this biomarker in combination with other genetic polymorphisms such as *GSTM1, GSTT1,* and *GSTP1* and DNA repair genes that may modulate the level of induced adducts will provide further information on the interaction between genes and BPDE exposure. Using a battery of complementary assays for genetic susceptibility to cancer may increase our ability to identify individuals at high risk and further our understanding of the underlying mechanisms of smoking-related carcinogenesis.

Table 6 *Multivariate logistic regression analysis of select variables and the level of BPDE-induced adducts in lung cancer cases and healthy controls*[a]

	Estimate (β) (OR; 95% CI)	χ^2	P
Intercept	−1.745		
Age (yr)	0.008 (1.01; 0.99–1.03)	0.421	0.517
Sex (male *vs.* female)	0.450 (1.57; 1.02–2.41)	4.181	0.041
Ethnicity (non-Hispanic white *vs.* others)	0.040 (1.04; 0.73–1.48)	0.048	0.827
Pack years	0.009 (1.01; 1.00–1.02)	6.511	0.010
Smoked in previous 24 h (yes *vs.* no)	0.120 (1.23; 0.60–1.92)	0.195	0.659
BMI	−0.062 (0.94; 0.90–0.99)	6.462	0.011
Weight loss (yes *vs.* no)	0.940 (2.56; 1.57–4.17)	14.341	<0.001
Family history of cancer (yes *vs.* no)	0.313 (1.37–1.01)	1.917	0.166
Level of DNA adducts/10^7 nt[b]	0.007 (1.01; 1.00–1.01)	17.305	<0.001

[a] This model included 195 cases and 219 controls because of missing data on BMI.
[b] nt, nucleotides.

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REFERENCES

1. American Cancer Society. Cancer facts and figures, 2000. Atlanta: American Cancer Society, 2000.
2. United States Surgeon General. Reducing the health consequences of smoking. Twenty-five years of progress. USPHS Publication No. (CDC), 89-8411. Rockville, MD: Department of Health and Human Services, 1989.
3. Shields, P. G., and Harris, C. C. Molecular epidemiology and the genetics of environmental cancer. J. Am. Med. Assoc., *266:* 681–687, 1991.
4. Perera, P. P. Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. J. Natl. Cancer Inst., *88:* 496–509, 1996.
5. Hecht, S. S., Carmella, S. G., Murphy, S. E., Foiles, P. G., and Chung, F. L. Carcinogen biomarkers related to smoking and upper aerodigestive tract cancer. J. Cell Biochem. Suppl., *17F:* 27–35, 1993.
6. Rebbeck, T. R. Molecular epidemiology of human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. Cancer Epidemiol. Biomark. Prev., *6:* 733–743, 1997.
7. Li, D., Wang, M., Cheng, L., Spitz, M. R., Hittelman, W. N., and Wei, Q. *In vitro* induction of benzo(*a*)pyrene diol epoxide-DNA adducts in peripheral lymphocytes as a susceptibility marker for human lung cancer. Cancer Res., *56:* 3638–3641, 1996.
8. Hudmon, K. S., Honn, S. E., Jiang, H., Chamberlain, R. M., Xiang, W., Ferry, G., Gosbee, W., and Spitz, M. R. Identifying and recruiting healthy control subjects from a managed care organization: a methodology for molecular epidemiologic case-control studies of cancer. Cancer Epidemiol. Biomark. Prev., *6:* 565–571, 1997.
9. Moutain, C. F. Revisions in the International System for Staging Lung Cancer. Chest, *111:* 1710–1717, 1997.
10. Barret, J. M., Calsou, P., and Salles, B. Deficient nucleotide excision repair activity in protein extracts from normal human lymphocytes. Carcinogenesis (Lond.), *7:* 1611–1616, 1995.
11. Wei, Q., Gu, J., Cheng, L., Bondy, M. L., Jiang, H., Hong, W. K., and Spitz, M. R. Benzo(*a*)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. Cancer Res., *56:* 3975–3979, 1996.
12. Venkatachalam, S., Denissenko, M., and Wani, A. A. DNA repair in human cells: quantitative assessment of bulky anti-BPDE-DNA adducts by non-competitive immunoassays. Carcinogenesis (Lond.), *16:* 2029–2036, 1995.
13. Reddy, M. V., and Randerath, K. Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis (Lond.), *7:* 1543–1551, 1986.
14. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res., *58:* 604–608, 1998.
15. Perera, F., Mayer, J., Jaretzki, A., Hearne, S., Brenner, D., Young, T. L., Fischman, H. K., Grimes, M., Grantham, S., Tang, M. X., Tsai, W-Y., and Santella, R. M. Comparison of DNA adducts and sister chromatid exchange in lung cancer cases and controls. Cancer Res., *49:* 4446–4451, 1989.
16. Gupta, R. C., Earley, K., and Sharma, S. Use of human peripheral blood lymphocytes to measure DNA binding capacity of chemical carcinogens. Proc. Natl. Acad. Sci. USA, *85:* 3513–3517, 1988.
17. Spitz, M. R., Wu, X., Jiang, H., and Hsu, T. C. Mutagen sensitivity as a marker of cancer susceptibility. J. Cell Biochem., *S25:* 80–84, 1996.
18. Wu, X., Gu, J., Hong, W. K., Lee, J. J., Amos, C. I., Jiang, H., Winn, R. J., Fu, K. K., Cooper, J., and Spitz, M. R. Benzo(*a*)pyrene diol epoxide and bleomycin sensitivity and susceptibility to cancer of upper aerodigestive tract. J. Natl. Cancer Inst., *90:* 1393–1399, 1998.
19. Jahnke, G. D., Thompson, C. L., Walker, M. P., Gallagher, J. E., Lucier, G. W., and DiAugustine, R. P. Multiple DNA adducts in lymphocytes of smokers and nonsmokers determined by ^{32}P-postlabeling analysis. Carcinogenesis (Lond.), *11:* 205–211, 1990.
20. Krolewski, B., Little, J. B., and Reynolds, R. J. Effect of duration of exposure to benzo(*a*)pyrene diol-epoxide on neoplastic transformation, mutagenesis, cytotoxicity and total covalent binding to DNA of rodent cells. Teratog. Carcinog. Mutagen., *8:* 127–136, 1988.
21. Wei, Q., and Spitz, M. R. The role of DNA repair capacity in susceptibility to lung cancer: a review. Cancer Metastasis Rev., *16:* 295–307, 1997.
22. Rudiger, H. W., Nowak, D., Hartmann, K., and Cerutti, P. Enhanced formation of benzo(*a*)pyrene: DNA adducts in monocytes of patients with a presumed predisposition to lung cancer. Cancer Res., *45:* 5890–5894, 1985.
23. Wei, Q., Matanoski, G. M., Farmer, E. R., Hedayati, M., and Grossman, L. DNA repair and aging in basal cell carcinoma: a molecular epidemiologic study. Proc. Natl. Acad. Sci. USA, *90:* 1614–1618, 1993.
24. Muscat, J. E., Richie, J. P., Jr., Thompson, S., and Wynder, E. L. Gender differences in smoking and risk for oral cancer. Cancer Res., *56:* 5192–5197, 1996.
25. Wiencke, J. K., Kelsey, K. T., Varkonyi, A., Semey, K., Wain, J. C., Mark, E., and Christiani, D. C. Correlation of DNA adducts in blood mononuclear cells with tobacco carcinogen-induced damage in human lung. Cancer Res., *55:* 4910–4914, 1995.
26. Wei, Q., Cheng, L., Amos, C. I., Wang, L. E., Guo, Z. Z., Hong, W. K., and Spitz, M. R. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiological study. J. Natl. Cancer Inst., *92:* 1764–1772, 2000.
27. Athas, A. F., Hedayati, M., Matanoski, G. M., Farmer, E. R., and Grossman, L. Development and field-test validation of an assay for DNA repair in circulating human lymphocytes. Cancer Res., *51:* 5786–5793, 1991.
28. Kraemer, K. H., Lee, M. M., Andrews, A. D., and Lambert, W. C. The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. Arch. Dermatol., *130:* 1018–1021, 1994.
29. Hoeijmakers, J. H. J. Human nucleotide excision repair syndromes: molecular clues to unexpected intricacies. Eur. J. Cancer, *30:* 1912–1921, 1994.
30. Modrich, P. Mismatch repair, genetic stability, and cancer. Science (Washington DC), *266:* 1959–1960, 1994.
31. Pero, R. W., Johnson, D. B., Miller, D. G., Zang, E., Markowitz, M., Doyle, G. A., Lung-Pero, M., Seidgard, J., Halper, M., and Miller, D. G. DNA repair synthesis in individuals with and without a family history of cancer. Carcinogenesis (Lond.), *10:* 693–697, 1989.
32. Yang, X., and Lippman, M. E. BRCA1 and BRCA2 in breast cancer. Breast Cancer Res. Treat., *54:* 1–10, 1999.

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